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(54) Title: PREVENTION AND TREATMENT OF RESTENOSIS BY LOCAL ADMINISTRATION OF DRUG

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B. FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93/12143 A1 (KABI PHARMACIA AB.) 24 June 1993 (24.06.1993), claims, entire document (Apolopoprotein A-I Milano and methods of use; cardio).	1-34
Y	US 6,80,422 A (WILLIAMS et al) 19 June 2000 (19.06.00), entire document.	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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PREVENTION AND TREATMENT OF RESTENOSIS BY LOCAL ADMINISTRATION OF DRUG

Background of the Invention

This application claims priority to U.S. Serial No. 60/326,379 filed September 28, 2001.

The present invention is generally in the area of methods and compositions to reduce restenosis after revascularization of diseased coronary, peripheral, and cerebral arteries, and stenosis or restenosis of surgically-placed bypass grafts or transplanted organs, specifically by local administration of an agent such as apolipoprotein A-I Milano alone or in combination with lipid formulations or other cholesterol lowering agents or lipid regulating agents.

Angioplasty, surgery and other vascular interventions are complicated by an accelerated arteriopathy characterized by rapid growth of cells into the lumen within a short period of time. This growth is often severe enough to jeopardize the blood flow to distal organs.

Vascular bypass surgery has been widely used to treat stenotic and occluded blood vessels, as when plaques develop on the surface of blood vessels in atherosclerosis. In bypass surgery, one or more healthy blood vessels are grafted into the stenotic/occluded vessels beyond the site of stenosis or occlusion to shunt blood around the stenotic or occluded vessel to re-establish a sufficient blood supply to the tissue whose blood supply is endangered by the stenosis or occlusion. This surgery often successfully revascularizes the endangered tissue.

Angioplasty has been developed as an alternative treatment to bypass surgery, especially in patients who

have been diagnosed early in the development of stenosis or occlusion of blood vessels due to the abnormal laying down of plaque on the luminal wall of a blood vessel. Angioplasty typically involves guiding a catheter which is usually fitted with a balloon or expandable metal mesh to an artery region of stenosis or occlusion and the brief inflation, one or more times, of the balloon or wire mesh to push the obstructing intravascular material or plaque up against the endothelial wall of the vessel, thereby compressing and/or breaking apart the plaque and reestablishing blood flow. However, angioplasty treatment can injure the vessel, especially when the balloon is over inflated or the mesh overextended, causing a variety of undesirable results, such as denudation (removal) of the endothelial cell layer in the region of the angioplasty, dissection of part of the inner vessel wall from the remainder of the vessel with accompanying occlusion of the vessel, or rupture of the tunica intima layer of the vessel.

Injury of arteries in animals induces a process of vascular repair which eventually causes the artery to become narrowed. A thick new layer, or neointima, of smooth muscle cells and inflammatory cells grows within the blood vessel, encroaching on the lumen. This process in animals represents the process that occurs clinically after angioplasty, endovascular stent implantation, organ transplantation, or bypass surgery, which greatly limits the long term successes of these techniques for treating obstructive arterial disease. Animal models of arterial injury and neointimal hyperplasia have been used to study the cellular events which lead to restenosis in humans, to devise treatment strategies to suppress tissue growth in an

attempt to reduce restenosis and enhance long term clinical results. Pigs are particularly useful as an animal model for restenosis in humans.

Attempts to limit stenosis or restenosis of blood vessels following revascularization have included administration of pharmacologic agents and technical approaches. No pharmaceutical agent has been clinically approved for the indication to prevent restenosis in humans. One technical approach, endovascular stent placement, has been shown to partially reduce restenosis in humans after coronary arterial intervention, as reported by Serruys, et al. N. E. J. Med. 1994; 331:489-495 and Fischman, et al. N. E. J. Med. 1994; 331:496-501. Nevertheless, stents themselves remain susceptible to significant restenosis in 20 - 30% of cases.

Increased knowledge of the mechanisms underlying vascular repair has led to innovative proposals for agents to limit accelerated arteriopathies. Circulatory leukocytes, including monocytes, are known to be among the very first cells recruited to blood vessels as atherosclerosis begins. Once within diseased arterial walls, these cells may engulf cholesterol and other lipids, and may also produce substances that attract other cells, cause other cells to proliferate, or degrade matrix components. Each of these secondary effects may in turn promote greater intimal thickening and more severe narrowing or occlusion of the arterial lumen. A similar role for leukocytes in restenosis after revascularization has not been proven. Although leukocyte activation has been connected to restenosis in humans (Pietersma, et al. Circulation 1995; 91:1320-1325; Mickelson, et al., 1996 JACC 28(2):345-353; Inoue, et al.,

1996 JACC 28(5):1127-1133) broad inhibition of inflammation, for example with glucocorticoids, after revascularization has not reduced restenosis in humans (Pepine et al., Circulation 1990; 81:1753-1761). This observation is reminiscent of studies using both broadly active and very specifically targeted treatments for preventing restenosis. Broad treatments, for example with heparin, have been limited by systemic toxicities and dosing limitations. Specific treatments, for example with molecular strategies, have failed to inhibit all of the redundant cellular and molecular pathways which activate and potentiate the vascular repair process.

Ameli, et al., Circulation 90(4):1935-41 (1994) reported that several epidemiological studies have shown an inverse relation between high-density lipoprotein (HDL) cholesterol levels and coronary heart disease and a similar inverse relation between HDL and restenosis after coronary balloon angioplasty. They conducted a study to determine whether HDL directly influences neointima formation, investigating the effect of recombinant apoA-I Milano (apoA-IM, a variant of human apoA-I with Arg-173 to Cys substitution), on intimal thickening after balloon injury in cholesterol-fed rabbits. Rabbits received intravenous injections of 40 mg of apoA-IM linked to a phospholipid carrier on alternate days, beginning 5 days before and continuing for 5 days after balloon injury of femoral and iliac arteries (a total dosage of 200 mg/animal, or about 11.4 mg/kg/dose). Three weeks after balloon injury, apoA-IM-treated rabbits had significantly reduced intimal thickness compared with the two control groups. The intima-to-media ratio was also significantly reduced by apoA-IM by ANOVA

compared with the two controls. The fraction of intimal lesion covered by macrophages, as identified by immunohistochemistry using a macrophage-specific monoclonal antibody, was significantly less in apoA-IM-treated rabbits compared with carrier-treated animals (25.3 +/- 17% versus 59.4 +/- 12.3%, $P < .005$). Aortic cholesterol content, did not differ significantly between apoA-IM-treated animals and carrier alone-treated controls. Unfortunately, unlike in pigs where the lesions are more human like, results obtained in rabbits have not been predictive of results in humans.

Accordingly, there is a need for compositions and methods of promoting healing of vascular tissue and controlling vascular muscle cell proliferation (hyperplasia) to prevent restenosis of blood vessels after angioplasty, vascular bypass, organ transplantation, or vascular disease, with minimal risk of rapid reocclusion.

It is therefore an object of the present invention to provide a method and compositions to reduce restenosis after revascularization of diseased coronary, peripheral, and cerebral arteries and stenosis or restenosis of surgically-placed bypass grafts or transplanted organs.

It is a further object of the present invention to provide a method which is a simple and effective means of gene transfer.

Summary of the Invention

Apolipoprotein A-I (ApoA-I), preferably a variant form such as Apolipoprotein A-I Milano (ApoA-IM), alone or more preferably in combination with a lipid carrier such as phospholipids or other drug, can be administered locally before or during bypass surgery on diseased coronary,

peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, or angioplasty, or to stabilize unstable plaques. In the preferred embodiment, ApoA-IM is administered using an INFILTRATOR, intramural delivery device and/or other sustained, controlled release means of administration of the ApoA-IM, so that an effective dose is administered at the site of injury. Based on a pig model using ApoA-IM, an effective dose for treatment or prevention of restenosis is in the range of from 0.2 to 0.4 mg ApoA-IM/kg delivered to the site to be treated, or more specifically between 4 and 6 mg ApoA-IM/vessel to be treated. The upper dosage for intramural administration by INFILTRATOR is limited by the viscosity of the solution. For example, the method cannot be used with a solution of ApoA-IM which is too viscous to pass through the pores of an INFILTRATOR. As demonstrated by the examples, an effective amount is in the range of 0.3 to 0.4 ml of a 14 mg/ml solution of ApoA-IM (which is equivalent to between 4 and 6 mg in a single dose/vessel segment or about a dose of about 0.2 mg/kg for each vessel treated in a 25 kg pig), preferably administered in combination with 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) at a ratio of about 1:1 by weight.

In an alternative embodiment, the apolipoprotein is not provided directly, but the gene encoding the apolipoprotein is provided. The gene is introduced into the blood vessel in a manner similar to that used for the protein, where the protein is then expressed. The technique can also be used for delivery of genes for treatment or prevention or restenosis or other cardiovascular diseases.

In yet another embodiment, stents are coated with apolipoproteins alone, apolipoproteins formulated with lipids, genetically engineered cells expressing the apolipoproteins, naked DNA coding for an apolipoprotein, or other drugs such as anti-proliferatives for local delivery to an injury site. This embodiment also includes instances whereby coatings are added in combination to stents to allow greater effectiveness. In a preferred embodiment, the system is used with combination therapy, with for local delivery of an agent such as an apolipoprotein in combination with systemic antihypertension therapy, lipid regulation and/or anti-coagulation therapy. Examples of drugs that can be utilized include lipid regulating agents such as niacin, statins, and fibrates; agents for glycemic control; anti-hypertensive agents; and agents that prevent or delay blood coagulation or platelet aggregation such as where the agent is aspirin, IIb/IIIa inhibitors, cloprigel or heparin. Maximum benefit may be obtained using local delivery therapy with more than one combination- e.g. local delivery plus anticoagulation plus lipid regulation. These treatments could begin prior to, concurrent with or following local delivery. Preferably the systemic treatments would be begun prior to the local delivery procedure.

Detailed Description of the Drawings

Figures 1-4 relate to tests in which ten domestic pigs were treated once with a single intravenous infusion of 100 mg/kg ETC-216, an apoA-IM/POPC (approximately 1/1 by weight) complex (n=5) or saline (n=5). Test agents were administered intravenously over approximately 3 hours while undergoing overstretch percutaneous transluminal coronary angioplasty with stent deployment in two coronary

vessels per animal. Dosage was based on the weight of the protein component of the complex. Animals were euthanized on day 28 (8 animals) or day 29 (one animal) following quantitative coronary angiography and intravascular ultrasound (IVUS), coronary arteries were perfused and fixed for histomorphometric analysis. One control animal expired on day 27, and only histomorphometric data was collected and used from this animal.

Figures 1a and 1b are graphs of the quantitative coronary angiographic data (QCA), which was determined at three time points, prior to coronary injury, immediately following coronary injuries and stent deployment, and prior to sacrifice, and are based on diameter measurements (mm) made at an unstented segment proximal to the stent, at the proximal section of the stent , an average area throughout the length of the stent , at the distal section of the stent , and at an unstented segment distal to the stent. From these data, maximum diameter and minimum diameter of the stented areas were determined. QCA estimation of lumen gain (luminal diameter post-injury minus pre-injury) and lumen loss (luminal diameter post-injury minus 28-29-day follow-up) were determined in the stented and adjacent unstented segments. The QCA data for all vessels (i.e. the right coronary artery (RCA) and left anterior descending artery (LAD) combined) are shown in Figure 1a and for vessel type (i.e. the RCA or the LAD) in Figure 1b.

Figures 2a and 2b are graphs of the intravascular ultrasound (IVUS) data used to determine stent and lumen area at the distal, middle and proximal region of each stented coronary vessel prior to sacrifice (28-29 days post-surgery). The difference between these measurements is the

neointimal area. Figure 2a is for all vessels (i.e. the RCA and LAD combined) and Figure 2b is for vessel type (i.e. the RCA or the LAD).

Figures 3a and 3b are the histomorphometric analysis of stented arteries used to determine the average cross sectional areas of the adventitial boundary layer (ABL), the external elastic lamina (EEL), the internal elastic lamina (IEL), the lumen (L), the adventitia (A), the media (M), the intima (I), the intima to media ratio (I/M) and an injury score. The injury score is the average of 36 determinations of injury consisting of twelve determinations in each of the proximal (a), middle (b) and distal (c) segments at the strut sites of the stented vessel. Injuries were scored either 0,1, 2 or 3 with 0 indicating an intact IEL (i.e. no injury) and 3 indicating a ruptured EEL with exposure to the adventitia (i.e. most severe injury). Figure 3a is the histomorphometric data for all vessels (i.e. the RCA and LAD combined) and Figure 3b is the histomorphometric data for vessel type (i.e. the RCA or the LAD).

Figure 4 shows select correlations between histomorphometric and IVUS variables.

Figure 5a and b show the histomorphometric data collected from domestic pigs administered a 0.3-0.4 ml solution containing 4-6 mg protein/vessel ETC-216, an apoA-IM/POPC (~1/1 by weight) complex (n=6 pigs) or sucrose-mannitol vehicle (n=6 pigs) using an INFILTRATOR®, an intramural delivery device, with subsequent overstretch percutaneous transluminal coronary angioplasty with stent deployment in two coronary vessels per animal. Dose represents the weight of the protein

component in the complex. Shown are the histomorphometric analysis of stented arteries used to determine the average cross sectional areas of the adventitial boundary layer (ABL), the external elastic lamina (EEL), the internal elastic lamina (IEL), the lumen (L), the adventitia (A), the media (M), the intima (I), the intima to media ratio (I/M) and the injury score. The injury score is the average of 36 determinations of injury consisting of twelve determinations in each of the proximal (a), middle (b) and distal (c) segments at the strut sites of the stented vessel. Injuries were scored either 0,1, 2 or 3 with 0 indicating an intact IEL (i.e. no injury) and 3 indicating a ruptured EEL with exposure to the adventitia (i.e. most severe injury). The histomorphometric data of all vessels (i.e. the RCA, left circumflex (LCX) and LAD combined) is shown in Figure 5a and by vessel type individually (i.e. the RCA, LCX or the LAD) is shown in Figure 5b.

Description of the Invention

The system that has been developed is focused on the local application of a material that is useful in treating or preventing restenosis, locally before or during bypass surgery on diseased coronary, peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, or angioplasty, or to stabilize unstable plaques. The local administration is preferably achieved using a device that includes a reservoir that slowly releases drug over a period of time. The reservoir may be a part of the device, such as a stent, or created by injection into a particular tissue or organ, for example, via intrapericardial or INFILTRATOR administration. This can be done using commercially

available cathethers.

The compositions to be administered may be cholesterol and oxidized lipid removing agents (such as apolipoproteins in combination with phospholipid, statins, fibrates), DNA encoding such agents (for example, encoding the apolipoproteins) or other proteins such as enzymes involved in nitric oxide generation, and/or drugs such as anti-proliferative compounds like rapamycin, paclitaxel or antibodies such as tirofiban and abciximab.

Combination therapy may also be used where drug such as ApoA-IM is administered locally and another drug is administered systemically, for example, systemic anti-hypertension therapy, lipid regulation and/or anti-coagulation therapy. Examples of drugs that can be utilized include lipid regulating agents such as niacin, statins, and fibrates; agents for glycemic control; anti-hypertensive agents; and agents that prevent or delay blood coagulation or platelet aggregation such as where the agent is aspirin, IIb/IIIa inhibitors, clopidogrel or heparin.

I. Lipid Modulating Agents

Apolipoprotein Formulations

Compounds which function as HDL include synthetic HDL which contains lipid such as phosphotidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, and other phospholipids in combination with HDL associated proteins such as apoA-I or variants thereof including apoAI-Milano and biologically active peptides derived therefrom, reverse lipid transport (RLT) peptides, enzymes associated with HDL such as paraoxonase, and apo E, alone or formulated in combination with liposomes or emulsions. As used herein, HDL associated proteins include sequences present

in HDL associated proteins that associate with HDL and synthetic peptides having equivalent binding or functional characteristics. Compounds which enhance HDL function include liposomes, where the HDL acts as a shuttle from the cells to the liposome. Suitable liposomal formulations are described in WO 95/23592 by the University of British Columbia.

The formulations described herein typically consist of an alpha helical protein such as an ApoA-I, a lipid, and a carrier.

ApoA-I and ApoA-IM are representative compositions that can be used to treat or prevent stenosis arising as a result of bypass surgery on diseased coronary, peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, or angioplasty.

Plasma ApoA-I is a single polypeptide chain of 243 amino acids, whose primary sequence is known (Brewer et al, Biochem. Biophys. Res. Commun. 80:623-630 (1978)). ApoA-I is synthesized as a 267 amino acid precursor in the cell. This preproapolipoproteinA-I is first intracellularly processed by N-terminal cleavage of 18 amino acids to yield proapolipoproteinA-I, and then further cleavage of 6 amino acids in the plasma or the lymph by the activity of specific proteases to yield apolipoproteinA-I. The major structural requirement of the ApoA-I molecule is believed to be the presence of repeat units of 11 or 22 amino acids, presumed to exist in amphipathic helical conformation (Segrest et al, FEBS Lett 38:247-253 (1974)). This structure allows for the main biological activities of ApoA-I, i.e. lipid binding and lecithin:cholesterol acyltransferase (LCAT) activation.

Human apolipoprotein AI-Milano (ApoA-IM) is a natural variant of ApoA-I (Weisgraber et al. J. Clin. Invest 66: 901-907 (1980)). In ApoA-IM the amino acid Arg173 is replaced by the amino acid Cys173. Since ApoA-IM contains one Cys residue per polypeptide chain, it may exist in a monomeric, homodimeric, or heterodimeric form. These forms are chemically interchangeable, and the term ApoA-IM does not, in the present context, discriminate between these forms. On the DNA level the variant form results from a C to T substitution in the gene sequence, i.e. the codon CGC changed to TGC, allowing the translation of a cys instead of arg at amino acid position 173. However, this variant of ApoA-I is one of the most interesting variants, in that ApoA-IM subjects are characterized by a remarkable reduction in HDL-cholesterol level, but without an apparent increased risk of arterial disease (Franceschini et al. J. Clin. Invest 66: 892-900 (1980)).

Another useful variant of ApoA-I is the Paris variant, where the arginine 151 is replaced with a cysteine.

The systemic infusion of ApoA-I alone (Miyazaki et al. *Arterioscler Thromb Vasc Biol.* 15:1882-1888(1995) or of HDL (Badimon et al, *Lab Invest.* 60:455-461 (1989) and *J Clin Invest.* 85:1234-1241 (1990)) in experimental animals and initial human clinical studies (Nanjee et al., *Arterioscler Thromb Vasc Biol.* 19:979-989(1999) and Eriksson et al. *Circulation.* 100:594-598 (1999)) has been shown to exert significant biochemical changes, as well as to reduce the extent and severity of atherosclerotic lesions. It has now been discovered that it can be administered locally at a site of injury, and significantly reduce stenosis or restenosis, as

discussed in more detail below and demonstrated by the following examples.

Other HDL-associated apolipoproteins with alpha-helical characteristics could be used. Examples include Apo E, proApoA-I, ApoA-IParis, ApoA-II, proApoA-II, ApoA-IV, ApoC-I, ApoC-II, and ApoC-III, the alpha-helical sequences within these proteins, and apolipoproteins modified to include one or more sulfhydral groups, as described by Bielicki and Oda, *Biochemistry* 41:2089-2096 (2002). Additional HDL associated proteins can be used. Examples include paraoxonase, cholesteryl ester transfer protein, LCAT and phospholipid transfer protein. The above proteins can be used alone, in combination, complexed to lipid alone or in combination complexed to lipid. In addition, mixtures of complexes can be useful. An example is complexes comprised of ApoA-I with lipid and complexes comprised of paraoxanase with lipid administered as a mixture. Another example includes complexes comprised of greater than one protein component. For example, complexes comprised of ApoA-I, paraoxonase and lipid are useful.

Lipids

Lipids form a complex with the ApoA-I which enhances its efficacy. Typically, the lipid is mixed with the ApoA-I prior to administration. Apolipoprotein and lipids are mixed in an aqueous solution in appropriate ratios and can be complexed by methods known in the art and including freeze-drying, detergent solubilization followed by dialysis, microfluidization, sonication, and homogenization. Complex efficiency can be optimized, for example, by varying pressure, ultrasonic frequency, or detergent

concentration. An example of a detergent commonly used to prepared apolipoprotein-lipid complexes is sodium cholate.

In some cases it is desirable to mix the lipid and the apolipoprotein prior to administration. Lipids may be in solution or in the form of liposomes or emulsions formed using standard techniques such as sonication or extrusion. Sonication is generally performed with a tip sonifier, such as a Branson tip sonifier, in an ice bath. Typically, the suspension is subjected to several sonication cycles. Extrusion may be carried out by biomembrane extruders, such as the Lipex Biomembrane Extruder. Defined pore size in the extrusion filters may generate unilamellar liposomal vesicles of specific sizes. The liposomes may also be formed by extrusion through an asymmetric ceramic filter, such as a Ceraflow Microfilter, commercially available from the Norton Company, Worcester Mass. or through a polycarbonate filter or other types of polymerized materials (i.e. plastics) commonly known.

In some cases it is preferable to administer the apolipopotein alone, essentially lipid-free, to treat the injured artery. The aqueous sterile solution is added to the apolipoprotein. The apolipoprotein in solution can be administered to treat an injured artery.
Alternative, freeze-dried preparation of complexes may be hydrated with an aqueous solution prior to administration. In other cases, frozen preparations of complexes in aqueous solution are thawed until a homogenous solution is achieved prior to administration to an injured vessel,

Preferred lipids are phospholipids, most preferably including at least one phospholipid, typically soy phosphatidylcholine, egg phosphatidylcholine, soy

phosphatidylglycerol, egg phosphatidylglycerol, palmitoyl-oleoyl-phosphatidylcholine distearoylphosphatidylcholine, or distearoylphosphatidylglycerol. Other useful phospholipids include, e.g., phosphatidylcholine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, phosphatidic acid, N-(2,3-di(9-(Z)-octadecenyloxy))-prop-1-yl-N,N,N-trimethylammonium chloride, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylinositol, cephalin, cardiolipin, cerebrosides, dicetylphosphate, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, dioleoylphosphatidylglycerol, stearoyl-palmitoyl-phosphatidylcholine, di-palmitoyl-phosphatidylethanolamine, distearoyl-phosphatidylethanolamine, dimyrstoyl-phosphatidylserine, and dioleyl-phosphatidylcholine. Non-phosphorus containing lipids may also be used, including stearylamine, docecylamine, acetyl palmitate, and fatty acid amides.

Additional lipids suitable for use are well known to persons of skill in the art and are cited in a variety of well known sources, e.g., McCutcheon's Detergents and Emulsifiers and McCutcheon's Functional Materials, Allured Publishing Co., Ridgewood, N.J. Generally, it is desirable that the lipids are liquid-crystalline at 37°C, 35 °C, or 32 °C. Lipids in the liquid-crystalline state typically accept cholesterol more efficiently than lipids in the gel state. As patients typically have a core temperature of about 37 °C, lipids that are liquid-crystalline at 37 °C are generally in a liquid-crystalline state during treatment.

The concentration of the lipid in the formulation may vary. Persons of skill may vary these concentrations to optimize treatment with different lipid components or of particular patients. ApoAI is combined with lipid in a ratio by weight of between 1:0.5 to 1:3, with more lipid being preferred for clearance of cholesterol. A ratio of around 1:1 is preferred to produce the most homogenous population and for purposes of producing stable and reproducible batches.

Other Lipid Modulating Drugs

Compounds can also be administered with compounds that increase HDL levels specifically (i.e., not as a byproduct of decreasing LDL), and thereby improve the HDL cholesterol to total cholesterol ratio, and administration of combinations of any of these which are effective to improve the HDL to total blood cholesterol levels.

Examples of drugs include lipid regulating agents such as niacin, statins, and fibrates.

Anti-Proliferative Drugs

The infiltrator can also be used for delivery of agents such as anti-proliferatives like paclitaxel and topotecan (*Biochemical Pharmacology*, 2001;61(1):119-127).

Gene Delivery

In an alternative embodiment, genes encoding a protein to be delivered may be administered, rather than the protein. Gene transfer can be obtained using direct transfer of genetic material, in a plasmid or viral vector, or via transfer of genetic material in cells or carriers such as cationic liposomes. Such methods are well known in the art and readily adaptable for use in the gene mediated toxin therapies described herein. As reviewed by Francis, et al. Am. J. Pharmacogenomics 1(1):55-66 (2001), gene therapy

offers a novel approach for prevention and treatment of cardiovascular diseases. Technical advances in viral vector systems and the development of fusigenic liposome vectors have been crucial to the development of effective gene therapy strategies directed at the vasculature and myocardium in animal models. Gene transfer techniques are being evaluated as potential treatment alternatives for both genetic (familial hypercholesterolemia) and acquired occlusive vascular diseases (atherosclerosis, restenosis, arterial thrombosis) as well as for cardiac disorders including heart failure, myocardial ischemia, graft coronary arteriosclerosis and hypertension. See also, Teiger, et al., J. Cardiovasc. Pharmacol. 33(5):726-732 (1999).

Studies by Wolff et al., Biotechniques 11: 474-85 (1991), demonstrate injection of naked DNA into muscle allows long term and low expression levels of proteins coded for within the DNA sequence. Administration of naked DNA to smooth muscle layers can be achieved by use of an intramural device, such as an INFILTRATOR® and allow expression of the proteins or their alpha helical domains to treat the injured vessel. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)). Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, **247**, 1465-1468, (1990); and Wolff, J. A. Nature, **352**, 815-818, (1991). As used herein, plasmid or viral vectors are agents that transport the gene

into a cell without degradation and include a promoter yielding expression of the gene in the cell into which it is delivered. In a preferred embodiment vectors are derived from either a virus or a retrovirus. Preferred viral vectors are Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also preferred are any viral families which share the properties of these viruses which make them suitable for use as vectors. Preferred retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector.

Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not useful in non-proliferating cells. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In MICROBIOLOGY-1985, American Society for Microbiology, pp. 229-232, Washington, (1985). Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)).

Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. The construction of replication-defective adenoviruses has been

described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, *in vivo* delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396

(1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

Viral vectors have higher transaction (ability to introduce genes) abilities than do most chemical or physical methods to introduce genes into cells. Typically, viral vectors contain nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines that have been engineered to express the gene products of the early genes in trans.

The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function

when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements. Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of

transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

It is preferred that the promoter and/or enhancer region act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. It is further preferred that the promoter and/or enhancer region be active in all eukaryotic cell types. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTF. It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types.

Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of

transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In a preferred embodiment of the transcription unit, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

The viral vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure.

In a preferred embodiment, intramural delivery of DNA coding for ApoA-I, ApoA-IV, ApoE, paraoxonase or alpha-helical regions within these proteins are delivered to

an artery with or with out lipid to treat injured blood vessels.

DNA encoding a number of different proteins may also be delivered. For example, as described by Chen, et al., Jpn. J. Pharmacol. 89(4):327-336 (2002), cardiovascular gene transfer is not only a powerful technique for studying the function of specific genes in cardiovascular biology and pathobiology, but also a promising strategy for treating cardiovascular diseases. Since the mid-1990s, nitric oxide synthase (NOS), the enzyme that catalyzes the formation of nitric oxide (NO) from L-arginine, has received considerable attention as a potential candidate for cardiovascular gene therapy, because NO exerts critical and diverse functions in the cardiovascular system, and abnormalities in NO biology are apparent in a number of cardiovascular disease processes including cerebral vasospasm, atherosclerosis, postangioplasty restenosis, transplant vasculopathy, hypertension, diabetes mellitus, impotence and delayed wound healing. There are three NOS isoforms, i.e., endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). All three NOS isoforms have been used in cardiovascular gene transfer studies with encouraging results.

Kipshidze, et al., J. Am. Coll. Cardio. 39(10):1686-1691 (2002) describes decreasing neointimal formation by intramural delivery of antisense oligonucleotides.

Turunen, et al., Mol Ther 6(3):306 (2002), describes gene therapy with nuclear targeted lacZ- and TIMP-1-encoding adenoviruses were coupled to a peptide-motif (HWGF) that can bind to matrix metalloproteinase (MMP)-2 and MMP-9. In vivo, local intravascular catheter-mediated gene transfer of a HWGF-targeted TIMP-1-encoding

adenovirus (AdTIMP-1(HWGF)) significantly reduced intimal thickening in a rabbit aortic balloon denudation model compared with the control adenovirus.

The advantage of the method disclosed herein is that it provides for delivery and release over a much longer time period at the site in need of treatment.

Drugs for Systemic Treatment

A variety of different drugs can be administered systemically and/or locally. These include agents for glycemic control; anti-hypertensive agents; anti-inflammatory agents such as steroidal anti-inflammatory agents, cyclooxygenase-2 (COX-2) inhibitors, such as Celebrex, VIOXX, and cyclooxygenase inhibitors like ibuprofen and other non-steroidal anti-inflammatory agents, and agents that prevent or delay blood coagulation or platelet aggregation such as where the agent is aspirin, IIb/IIIa inhibitors, clopidogrel or heparin.

Stent Coatings

The stents may also be coated with apolipoproteins alone, apolipoproteins formulated with lipids, cells expressing the apolipoproteins or other proteins, DNA encoding the therapeutic proteins, or drugs having a local effect, such as paclitaxel, rapamycin or other anti-proliferative compounds. The coatings then release drug at the site of the injury, plaque, or area to be treated.

Pharmaceutically Acceptable Carriers

The pharmaceutical compositions typically include a pharmaceutically acceptable carrier. Many pharmaceutically acceptable carriers may be employed. The examples utilize sucrose-mannitol. Generally, normal saline will be employed as the pharmaceutically acceptable

carrier. Other suitable carriers include glucose, trehalose, sucrose, sterile water, buffered water, 0.4% saline, and 0.3% glycine, and can further include glycoproteins for enhanced stability, such as albumin, apolipoprotein, lipoprotein, globulin, etc. These compositions may be sterilized by conventional, well known sterilization techniques. The resulting aqueous solutions may be packaged for use or filtered under aseptic conditions and lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, and tonicity adjusting agents, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, and calcium chloride.

In another embodiment, the ApoA-I is administered in a gel, or a polymer solution that forms a gel at the site of administration. In one embodiment, calcium alginate and certain other polymers that can form ionic hydrogels which are malleable. For example, a hydrogel can be produced by cross-linking the anionic salt of alginic acid, a carbohydrate polymer isolated from seaweed, with calcium cations, whose strength increases with either increasing concentrations of calcium ions or alginate. The alginate solution is mixed with the ApoA-I to form an alginate suspension which is injected directly into a patient prior to hardening of the suspension. The suspension then hardens over a short period of time due to the presence in vivo of physiological concentrations of calcium ions. Modified alginate derivatives, for example, more rapidly degradable or which

are derivatized with hydrophobic, water-labile chains, e.g., oligomers of (-caprolactone, may be synthesized which have an improved ability to form hydrogels. Additionally, polysaccharides which gel by exposure to monovalent cations, including bacterial polysaccharides, such as gellan gum, and plant polysaccharides, such as carrageenans, may be crosslinked to form a hydrogel using methods analogous to those available for the crosslinking of alginates described above. Additional examples of materials which can be used to form a hydrogel include polyphosphazines and polyacrylates, which are crosslinked ionically, or block copolymers such as PluronicsTM or TetronicsTM, polyethylene oxide-polypropylene glycol block copolymers which are crosslinked by temperature or pH, respectively. Other materials include polymers such as polyvinylpyrrolidone, hyaluronic acid and collagen. Polymers such as polysaccharides that are very viscous liquids or are thixotropic, and form a gel over time by the slow evolution of structure, are also useful. For example, hyaluronic acid, which forms an injectable gel with a consistency like a hair gel, may be utilized. Modified hyaluronic acid derivatives are particularly useful. Polymer mixtures also may be utilized. For example, a mixture of polyethylene oxide and polyacrylic acid which gels by hydrogen bonding upon mixing may be utilized. In one embodiment, a mixture of a 5% w/w solution of polyacrylic acid with a 5% w/w polyethylene oxide (polyethylene glycol, polyoxyethylene) 100,000 can be combined to form a gel over the course of time, e.g., as quickly as within a few seconds.

Covalently crosslinkable hydrogel precursors also are useful. For example, a water soluble polyamine, such as

chitosan, can be cross-linked with a water soluble diisothiocyanate, such as polyethylene glycol diisothiocyanate. The isothiocyanates will react with the amines to form a chemically crosslinked gel. Aldehyde reactions with amines, e.g., with polyethylene glycol dialdehyde also may be utilized. A hydroxylated water soluble polymer also may be utilized.

Alternatively, polymers may be utilized which include substituents which are crosslinked by a radical reaction upon contact with a radical initiator. For example, polymers including ethylenically unsaturated groups which can be photochemically crosslinked may be utilized, as disclosed in WO 93/17669. Additionally, water soluble polymers which include cinnamoyl groups which may be photochemically crosslinked may be utilized, as disclosed in Matsuda et al., ASAID Trans., 38:154-157 (1992).

The gel material is applied either by spraying (in an open procedure) or by use of the INFILTRATOR or catheter (during a closed procedure). Typically, the ApoA-I alone, ApoA-I in combination with the lipid, or ApoA-I complexed with lipid is mixed with these gels at the time of solidification or polymerization, and then diffuses slowly out at the surface of the vessel being treated.

In another embodiment, the pharmaceutically acceptable carrier is coated on a stent. The carrier can be selected to release the invention in a time dependent fashion. Persons of skill may vary the carriers to achieve optimized stent coatings to achieve time dependent release of the invention from the stent.

II. Methods of Treatment

In the principle embodiment, a cholesterol-lowering agent such as Apolipoprotein A-I (ApoA-I), preferably a variant form such as Apolipoprotein A-I Milano (ApoA-IM), alone or more preferably in combination with a lipid carrier such as phospholipids or another drug, is administered locally using a reservoir device such as an INFILTRATOR® before or during bypass surgery on diseased coronary, peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, or angioplasty, or to stabilize unstable plaques, so that an effective dose is administered at the site of injury. In other embodiments, the same techniques and materials are administered to reduce the consequence of plaque rupture including thrombus formation and ischemia.

In other preferred embodiments, the local therapy is initiated in combination with systemic therapy, for example, in combination with agents to reduce restenosis, decrease or prevent plaque rupture, lower blood cholesterol, lower atherosclerotic lesion cholesterol, reduce blood coagulation, regulate one or more of the blood lipids (i.e,, lipid-regulating agent), reduce inflammation, or control blood pressure. Examples of drugs that can be utilized include lipid regulating agents such as niacin, statins, and fibrates; agents for glycemic control; anti-hypertensive agents; and agents that prevent or delay blood coagulation or platelet aggregation such as where the agent is aspirin, IIb/IIIa inhibitors, clopridogrel or heparin. These additional agents will typically be administered systemically at their normal therapeutic dosages.

Maximum benefit may be obtained using local delivery therapy with more than one combination- e.g. local delivery plus anticoagulation plus lipid regulation. These treatments can begin prior to, concurrent with or following local delivery. Preferably the systemic treatments would be begun prior to the local delivery procedure.

In the preferred embodiment, an Apo A-I formulation is administered by means of an intramural delivery device, such as the INFILTRATOR available from Intraventional Technologies, Inc, San Diego, CA, now owned by Boston Scientific. Another useful device is described by Pavlides, et al., Cathet. Cardiovasc. Diagn. 41(3):287-292 (1997). Other means of delivery can utilize catheters delivering the drug from a reservoir, either prior to angioplasty, during or after inflation of the balloon.

In the most preferred embodiment, an ApoA-IM formulation is administered in a single dose prior to or at the time of treatment. Treatments include angioplasty, bypass surgery of diseased coronary, peripheral, or cerebral arteries, implantation of vascular stents, implantation of transplanted organs or tissues, and stabilization of plaques.

The preferred dosage is determined through experimental studies, as was done in the following examples for ApoA-IM. Dosages for other apolipoproteins can be readily calculated based on the dosages for ApoA-IM, taking into account the differences in efficiency of cholesterol removal, half-lives, and other relevant pharmacokinetic parameters. Alternatively, the dosage for other apolipoproteins can be readily calculated by taking into account the differences in efficiency of the anti-oxidant, anti-inflammatory and anti-antithrombotic properties of the

preparations. The presence and amount of lipid can be similarly determined for the different formulations based on the experimental data obtained for ApoA-IM.

In general, the formulation is administered at the site of treatment. The actual total dosage when delivered locally is significantly less than the dosage that would have to be administered systemically to achieve the same local dosage, however, the local concentration is much higher than the previous studies in which the ApoA-I was administered systemically. As noted above, the preferred dosages for ApoA-IM are between 4 and 6 mg ApoA-IM/vessel(typically up to three segments are treated with a total dosage of around 4 to 18 mg ApoA-IM), or between about 0.05 and 0.3 mg ApoA-IM/kg body weight in a 70 kg mammal. The preferred ratio of protein to lipid is between 1:0.5 to 1:3, with more lipid being preferred for clearance of cholesterol, but a more equal amount of protein to lipid being preferred for purposes of stability and consistency of preparations for regulatory approval. Ratios of protein to lipid for preparations other than those containing apoA-IM are tested at various ratios of protein to lipid and the stability and consistency, and characteristics (such as complex size and cholesterol efflux capacity) are determined for regulatory approval.

Although a single administration has been demonstrated to be efficacious, multiple dosages can be administered. For example, intravenous administration at day –1, 0, 1, 2 and 3 of 20 mg ApoA-IM/kg body weight resulted in all balloon over-stretched injured vessels showing increased lumen area relative to controls four weeks after the procedure.

The present invention will be further understood by reference to the following non-limiting examples.

Percutaneous coronary interventions are now a major method to increase the lumen of narrowed blood vessels in patients with coronary ischemia. These procedures are performed by percutaneous transluminal coronary angioplasty (PTCA), commonly known as " ballooning". In most cases, the procedure is completed with deployment of a stent to the dilated area with the goal of increasing the diameter of the blood vessel to increase blood flow and relieve ischemia. A major drawback is the post-procedural closure of the blood vessel lumen known as restenosis. In the absence of stents, the post-procedural incidence of early recoil and thrombosis are problematic, and therefore most balloon procedures today also include deployment of a stent. Although stenting improves outcome, post-procedural neointimal growth of a stented vessel can cause restenosis and recurrent ischemia or other coronary events, including myocardial infarction. Therefore, methods to prevent neointimal growth of ballooned and stented blood vessels are desired to improve procedural outcome. The porcine model was selected as the appropriate test system for the purposes of this study. Evidence in the literature suggests that arterial dilatation and restenosis in the porcine model is similar to that of human restenosis. Hence, this model can be used for evaluating potential therapeutic agents for the treatment of clinical restenosis.

Example 1: Effect of single high dose intravenous delivery of ETC-216 , a preparation containing complexes comprised of ApoA-IM and palmitoyl-oleoyl-phosphatidylcholine (POPC) on restenosis in overstretch percutaneous transluminal coronary angioplasty (PTCA) with stent deployment in the coronary arteries of pigs.

The objective of this study was to determine the effect of a single intravenous drug delivery of ETC-216 during coronary PTCA and stent placement on restenosis in a porcine model of vascular injury.

Materials and Methods

Experimental Animals

The porcine model was selected as the appropriate test system for the purposes of this study. Evidence in the literature suggests that arterial dilitation and restenosis in the porcine model is similar to that of human restenosis. Hence, this model can be used for evaluation potential therapeutic agents for the treatment of restenosis. Animals were acclimated to the laboratory environment for a minimum 7 days and examined prior to initiation of the study to ensure that they appear healthy.

During the quarantine and prior to surgery the animals were housed individually in runs. Animal pens were cleaned twice daily. The temperature and humidity in the animals' quarters (70-78(F; 30-80% RH) were monitored to maintain a target range of 70-80°C and 30-80 percent relative humidity. The airflow in the room was sufficient to provide several exchanges per hour with 100% fresh filtered air. An automatic timing device provided an alternating 12-hour cycle of light and dark. Following surgery animals

were recovered in the recovery room, then returned to pens. Animals were fed once a day a meal of pig chow (Southwest Farms Hog Finisher Diet) obtained from Newco (Rancho Cucamonga, CA) throughout the experimental period, except on days of surgery, when animals were fasted overnight. Fresh water was supplied ad libitum via an automatic watering system.

Animals were randomly selected and assigned to two study groups. Additional animals were ordered for the study in the event that an animal died or had to be euthanized due to the surgical procedure or complications deemed to result from the surgical procedure.

Test substance consisted of either ETC-216, a recombinant apolipoprotein A-I Milano/1-palmitoyl-2oleoyl phosphatidylcholine (POPC) complex provided by Esperion Therapeutics, Inc., as a ready to inject solution or saline. ETC-216 solutions contain apoA-IM protein at about 14mg/ml with a protein to POPC ratio of about 1 to 1 by weight.

Intravenous administration was selected for this study, because it is the route intended for use in human clinical studies. The dose selection was based on animal body weight. Test substance were administered at a 100 mg/kg body weight dose. This dose was based on the apolipoprotein content of the complex. Thus, an average pig in the study would receive about 3000-3500 mg of drug. Control animals received saline.

Surgeries were performed in adult domestic swine (weighing approximately 30-35 kg, with one animal at 50kg). Animals were fed normal diet and housed in the vivarium. Swine were fasted overnight and pretreated with

oral aspirin, 325 mg, beginning 3 days prior to surgery and daily thereafter until euthanasia. Ticlopidine (250 mg) was given to animals beginning 3 days prior to surgery and daily for 14 days after surgery. After an overnight 16-hour fast, the animals were immobilized by an intramuscular (IM) injection of acepromazine (0.5 mg/kg), ketamine (20 mg/kg), and atropine (0.05 mg/kg); anesthesia was induced with intravenous (IV) thiopental (5-8 mg/kg); and maintained by 1-2% isoflorane after endotracheal intubation. Mechanical ventilation, arterial blood pressure (BP) and continuous electrocardiogram (ECG) monitoring were performed throughout the procedure. Animals were given cardizem (120 mg) daily for 2 days after surgery.

The surgical procedure consisted of exposure of a carotid artery followed by insertion of an 8F sheath into the carotid artery. The animals were given bretylium tosylate (250 mg IV), inderal (1 mg) and heparin (10,000 U IV) prior to coronary instrumentation. ETC-216 or saline was administered as an IV infusion commencing 90 minutes prior to the surgical procedure such that the entire dose was administered over approximately 3 hours. An 8F AL-0.75 guiding catheter was advanced to the ostia of the coronary arteries under fluoroscopic guidance. After administration of intracoronary nitroglycerin (200 mcg), angiography was performed in order to estimate the size of the vessel. Stent overstretch injury was performed in the first vessel, and then repeated in the second vessel. In all cases, stents were deployed in the LAD and RCA. Stents were deployed in the LAD and the RCA in segments averaging 2.7 to 3.0 mm in diameter using the location of diagonal or septal branches as anatomical reference. All stents were deployed using a

balloon that was inflated one to three times to 6-8 atmospheres for 30 seconds to obtain a final stent: artery ratio of ~ 1.3:1. Angiography was performed initially to target stent site and repeated to confirm injury at stent deployment site as evidenced by an obvious "step-up" and a "step-down" of the injured segment. The catheters were withdrawn, the carotid artery ligated and the skin incision closed. As prophylaxis against infection, all animals received antibiotics at the end of the procedure. The animals were recovered from anesthesia, returned to the vivarium, fed a normal chow diet with additional medication as described above.

Angiographic and IVUS Measurements

Quantitative coronary angiography (QCA) was used to assess mean and minimum luminal diameter at various time points, i.e., pre-injury, immediately post-injury and at 28-29 days follow-up prior to euthanasia.

Definition of terms used during Quantitative Coronary Angiography:

MLD = mean lumen diameter

R_1 = reference segment to proximal segment (unstented)

Prox. = proximal segment of the stented artery

Mid. = mid segment of the stented artery

Dist. = distal segment of the stented artery

R_2 = reference segment to distal segment (unstented)

Max = maximum luminal diameter throughout the stented segment

Min = minimum luminal diameter throughout the stented segment

Lumen gain = Luminal diameter post injury minus pre injury

Lumen loss = Luminal diameter post injury minus 28 days follow-up

Percent stenosis of the injured segments was estimated using the uninjured segments as reference. Stent area, lumen area, neointimal area, percent area stenosis parameters were measured by intravascular ultrasound (IVUS).

Late luminal loss was calculated as the difference in MLD immediately post-balloon injury and at 28-29 days follow-up, and the remodeling index was calculated as late luminal loss divided by post-injury MLD.

Analysis of Coronary Arteries at Follow-up

After 28 (n=8) or 29 (n=1) days, animals fasted overnight, were prepared for surgery as above, for follow-up angiography. (In one control saline-treated animal , that expired on day 27, only histology was performed). In addition, at follow-up, an IVUS catheter was deployed in the stented coronary arteries for IVUS study of each stented artery. Animals were then euthanized under anesthesia with IV pentobarbital 90mg/kg, and hearts excised after thoracotomy. The coronary arteries were perfused with saline to clear the blood and then perfusion-fixed with 2% paraformaldehyde for 15 minutes followed by immersion in 4% paraformaldehyde in phosphate buffer (pH 7.4) for 4 hours and finally stored in 70% ethanol. To preserve the integrity of the adventitia and perivascular tissues, coronary arteries were carefully removed along with adjacent tissues (the adipose tissue and the myocardium). For stented segments, special histologic processing was performed to maintain the vascular architecture with metallic struts in situ. Tissue blocks were embedded in

methyl methacrylate and cut with a diamond-wafering blade. Three radial cross sections containing 12 struts were cut: one from the proximal, one from the middle and one from the distal third of each stent. Sections were ground to a thickness of about 50 μm, optically polished, and stained with toluidine blue (paragon stain).

Histomorphometric analysis

A computerized imaging system (Image Pro Plus 4.0) was used for histomorphometric measurements of:

1. The mean cross sectional area and lumen thickness (area circumscribed by the intima/neointimal-luminal border); neointimal (area between the lumen and the internal elastic lamina, IEL, and when the IEL is missing, the area between the lumen and the remnants of media or the external elastic lamina, EEL); media (area between the IEL and EEL); vessel size (area circumscribed by the EEL but excluding the adventitial area); and adventitia (area between the periadventitial tissues, adipose tissue and myocardium, and EEL).

2. The injury score. To quantify the degree of vascular injury, a score based on the amount and length of tear of the different wall structures was used. The degree of injury was calculated as follows:

0 = intact IEL

1 = ruptured IEL with exposure to superficial medial layers

2 = ruptured IEL with exposure to deeper medial layers (medial dissection)

3 = ruptured EEL with exposure to the adventitia

Results

Five domestic pigs treated with saline or five pigs treated with ETC-216 (Four males and one female

pigs/group) were evaluated for post-treatment restenosis after 27-29 days.

Blood was obtained from some but not all animals over the course of the study for the determination of white blood cell count, red blood cell count, blood hemoglobin content, percent hematocrit, blood platelet counts. In all cases where these blood variables were determined, post-treatment values did not vary appreciably from the baseline values. That is, they were all within the normal range.

Heart rate and blood pressure were determined for all animals entered in the study at baseline. Heart rate and blood pressure were also periodically determined on most animals during the surgical procedures, and prior to sacrifice. For animals entered into the study these variables did not vary appreciably from baseline values due to either the surgical procedure or treatments.

Quantitative Coronary Angiography (QCA) was determined at three time points, prior to coronary injury, immediately following coronary injuries and stent deployment, and prior to sacrifice (**Figure 1a and Figure 1b**). Diameter measurements (mm) were made at an unstented segment proximal to the stent (R1), at the proximal section of the stent (Prox), an average area throughout the length of the stent (Aver), at the distal section of the stent (Dist), and at an unstented segment distal to the stent (R2). In addition, the maximum diameter (Max) and minimal diameter (Min) of the stented region were determined. QCA estimation of lumen gain and lumen loss were determined in the stented and adjacent unstented segments. Indexes for maximal (Lumen Loss Max Index) and minimal (Lumen Loss Min Index) of the stented vessels

were determined. The quantitative coronary angiographic data for all vessels (i.e. the RCA and LAD combined) or vessel type (i.e. the RCA or the LAD) are shown graphically in **Figures 1a and 1b**, respectively.

Intravascular ultrasound (IVUS) was used to determine stent and lumen area at the distal, middle and proximal region of each stented coronary vessel prior to sacrifice The difference between these measurements is the neointimal area. The averages of stent, lumen and neointimal area for each animal and segment were determined, and used to determine averages for treatment groups of the pooled (LAD plus RCA) or the individual coronary vessels (LAD or RCA). One control-treated pig died one day prior to its schedule procedure (i.e. on day 27) and therefore only histomorphometric measurements were made on its stented coronary vessels. The intravascular ultrasound data for all vessels (i.e. the RCA and LAD combined) and for vessel type (i.e. the RCA or the LAD) are shown graphically in **Figures 2a and 2b**, respectively.

Histomorphometric analysis of stented arteries were used to determine the average cross sectional areas of the adventitial boundary layer (ABL), the external elastic lamina (EEL), the internal elastic lamina (IEL), the lumen (L), the adventitia (A), the media (M), the intima (I), the intima to media ratio (I/M) and an injury score. The injury score is the average of 36 determinations of injury consisting of twelve determinations in each of the proximal (a), middle (b) and distal (c) segments at the strut sites of the stented vessel. Injuries were scored either 0,1, 2 or 3 with 0 indicating an intact IEL (i.e. no injury) and 3

indicating a ruptured EEL with exposure to the adventitia (i.e. most severe injury).

ETC-216 treatment significantly reduced the intimal to media (I/M) ratio in coronary vessels by 32 percent (RCA and LAD combined). This effect was largely due to a significant 38 percent reduction of the I/M ratio of the LAD and to a lesser extent a 22 percent reduction (not significant) in the RCA. It should be noted the RCA was significantly more resilient to injury (injury score = 1.87 ± 0.54) than the LAD (injury score = 2.57 ± 0.34). The histomorphometric data for all vessels (i.e. the RCA and LAD combined) and for vessel type (i.e. the RCA or the LAD) are shown graphically in **Figures 3a and 3b**, respectively. Select correlation between histomorphometric and IVUS variables are shown in **Figure 4**.

Example 2: Effect of INFILTRATOR intramural delivery of ETC-216 , a preparation containing complexes comprised of ApoA-IM and palmitoyl-oleoyl-phosphatidylcholine (POPC) on restenosis in overstretch percutaneous transluminal coronary angioplasty (PTCA) with stent deployment in the coronary arteries of pigs.

Materials and Methods

Experiments were performed in adult domestic swine weighing 25-30 kg. Animals were fed a normal chow diet for pigs and housed in the vivarium. Swine were fasted overnight and pretreated with oral aspirin (325 mg) beginning 3 days prior to surgery and daily thereafter until euthanasia. Ticlopidine (250 mg) was given to animals beginning 3 days prior to surgery and daily for 14 days after

surgery. Cardizem was given to animals (120 mg) daily for 2 days after surgery.

After an overnight 16 hour fast, the animals were immobilized by an intramuscular (IM) injection of acepromazine (0.5 mg/kg), ketamine (20 mg/kg), and atropine (0.05 mg/kg); anesthesia was induced with intravenous (IV) thiopental (5-8 mg/kg); and maintained by 1-2% isoflorane after endotracheal intubation. Mechanical ventilation, arterial blood pressure (BP) and continuous electrocardiogram (ECG) monitoring were performed throughout the procedure.

The surgical procedure consisted of exposure of a carotid artery and an 8F sheath inserted into the carotid artery. The animals were given bretylium tosylate (250 mg IV), inderal (1mg IV) and heparin (8,000 U IV) prior to coronary instrumentation. An 8F AL-0.75 guiding catheter was advanced to the ostia of the coronary arteries under fluoroscopic guidance. After administration of intracoronary nitroglycerin (200 mcg), quantitative coronary angiography was performed for all three coronary arteries in order to estimate the size of the vessels. The two largest vessels were selected for the procedure. ETC-216 or sucrose-mannitol vehicle was administered intramurally via INFILTRATOR during the surgical procedure prior to PTCA with stent deployment. The INFILTRATOR catheter was introduced for the delivery of ETC-216 or sucrose-mannitol vehicle at a very low dose to the coronary vessel wall to minimize the loss of the agent into the circulation. Two arteries in each animal were infiltrated with 4-6 mg ETC-216 or sucrose-mannitol vehicle each in a dose volume of 0.3-0.4 ml. Thus each animal received a total dose of about

8-12 mg ETC-216 localized to two artery segments. Each artery underwent infiltration procedure by inflating the attached balloon once to 1.5-2 atmospheres in pressure during the delivery of ETC-216 or sucrose-mannitol vehicle prior to balloon over-inflation with stent deployment. Following the infiltration procedure, stents were deployed precisely in the infiltrated segments of LAD, RCA, and LCX, using the location of diagonal or septal branches as anatomical reference. All stents were deployed using a balloon that were inflated one to three times to 6-8 atmospheres for 30 seconds to obtain a final stent: artery ratio of ~ 1.3:1. Angiography was performed initially to target stent site and repeated to confirm injury at stent deployment site as evidenced by an obvious "step-up" and a "step-down" of the injured segment. The catheters were withdrawn, the carotid artery ligated and the skin incision closed. As prophylaxis against infection, all animals received antibiotics at the end of the procedure. The animals were recovered from anesthesia, returned to the vivarium, fed a normal chow diet with additional medication as described above.

Angiographic and IVUS Measurements

Quantitative coronary angiography (QCA) was used to assess mean and minimum luminal diameter at various time points, i.e., pre-injury, immediately post-injury and at 28 days follow-up prior to euthanasia. Late luminal loss was calculated as the difference in MLD immediately post-balloon injury and at 28 days follow-up, and the remodeling index was calculated as late luminal loss divided by post-injury MLD.

Definition of terms used during Quantitative Coronary Angiography:

R1 = reference segment to proximal segment (unstented)

P = proximal segment of the stented artery

M = mid segment of the stented artery

D = distal segment of the stented artery

R2 = reference segment to distal segment (unstented)

Max = maximum luminal diameter throughout the stented segment

Min = minimum luminal diameter throughout the stented segment

Lumen gain = Luminal diameter post injury minus pre injury

Lumen loss = Luminal diameter post injury minus 28 days follow-up

Percent stenosis of the injured segments was estimated using the uninjured segments as reference. Stent area, lumen area, neointimal area, percent area stenosis (all IVUS) parameters were measured.

Analysis of Coronary Arteries at Follow-up

After 28 days, animals fasted overnight, were prepared for surgery as above, for follow-up angiography. In addition, at follow-up, an IVUS catheter was deployed in the stented coronary arteries for IVUS study of each stented artery. Animals were then euthanized under anesthesia with IV pentobarbital 90mg/kg, and hearts excised after thoracotomy. The coronary arteries were perfused with saline to clear the blood and then perfusion-fixed with 2% paraformaldehyde for 15 minutes followed by immersion in 4% paraformaldehyde in phosphate buffer (pH 7.4) for 4 hours and finally stored in 70% ethanol. To preserve the

integrity of the adventitia and perivascular tissues, coronary arteries were carefully removed along with adjacent tissues (the adipose tissue and the myocardium). For stented segments, special histologic processing was performed to maintain the vascular architecture with metallic struts in situ. Tissue blocks were embedded in methyl methacrylate and cut with a diamond-wafering blade. Three radial cross sections containing 12 struts were cut: one from the proximal, one from the middle and one from the distal third of each stent. Sections were ground to a thickness of about 50 um, optically polished, and stained with toluidine blue (paragon stain).

Histomorphometric analysis

A computerized imaging system Image Pro Plus 4.0 was used for histomorphometric measurements of:

1. The mean cross sectional area and lumen thickness (area circumscribed by the intima/neointimal-luminal border); neointimal (area between the lumen and the internal elastic lamina, IEL, and when the IEL is missing, the area between the lumen and the remnants of media or the external elastic lamina, EEL); media (area between the IEL and EEL); vessel size (area circumscribed by the EEL but excluding the adventitial area); and adventitia (area between the periadventitial tissues, adipose tissue and myocardium, and EEL).

2. The injury score. To quantify the degree of vascular injury, a score based on the amount and length of tear of the different wall structures was used. The degree of injury was calculated as follows:

0 = intact IEL

1 = ruptured IEL with exposure to superficial medial layers

2 = ruptured IEL with exposure to deeper medial layers (medial dissection)

3 = ruptured EEL with exposure to the adventitia

Results

Two coronary arteries each from fourteen domestic pigs were treated with sucrose-mannitol vehicle (control) or 4-6 mg ETC-216 (n=7 per group). Quantitative coronary angiography (QCA) of the left anterior descending (LAD), the left circumflex (LCX) and the right coronary arteries (RCA) was performed in order to estimate the size of each vessel; the two largest arteries were selected for the procedure. In each artery, the drug was delivered intramurally via the INFILTRATOR followed by overstretch percutaneous transluminal coronary angioplasty (PTCA) with stent deployment at the site of drug delivery. This surgical procedure induced a vascular injury in which inflammation, neointimal hyperplasia and restenosis develops. The stented arteries from all animals were subjected to QCA prior to, immediately following stenting, and just prior to sacrifice at day 28. In addition, IVUS was used to determine stent and lumen area to estimate the neointimal area just prior to sacrifice. After sacrifice, segments of the stented arteries were obtained for histomorphometric measurements to assess the extent of overstretch injury, the amount of neointimal hyperplasia, and restenosis. The coronary vessels from one vehicle-treated pig that expired nine days prior to its scheduled QCA and IVUS procedures was analyzed by histological methods only. One ETC-216-treated pig was euthanized six days after the scheduled twenty-eight days procedures due to an error in facility scheduling. In order to presume a

fair comparison, the data collected from these two pigs was excluded from the analysis.

In addition, two animals were treated with about a 3-fold concentrated preparation of ETC-216 delivered through the INFILTRATOR. The preparation was found to be too viscous to effectively deliver test agent through the device and was found to cause damage to the device balloon and therefore an increased amount of injury to the arteries limiting the use of device with viscous solutions.

Blood was obtained from all animals over the course of the study for the determination of white blood cell count, red blood cell count, blood hemoglobin content, percent hematocrit, blood platelet counts. In all cases where these blood variables were determined, post-treatment values did not vary appreciably from the baseline values. That is, they were all within the normal range. Heart rate and blood pressure were determined for all animals entered in the study at baseline. Heart rate and blood pressure were also periodically determined on most animals during the surgical procedures, and prior to sacrifice. For animals entered into the study these variables did not vary appreciably from baseline values due to either the surgical procedure or treatments.

Quantitative Coronary Angiography (QCA) was determined at three time points, prior to coronary injury, immediately following coronary injuries and stent deployment, and prior to sacrifice. Diameter measurements (mm) were made at an unstented segment proximal to the stent (R1), at the proximal section of the stent (Prox), an average area throughout the length of the stent (Aver), at the distal section of the stent (Dist), and at an unstented

segment distal to the stent (R2). Intravascular ultrasound (IVUS) was used to determine stent and lumen area at the distal, middle and proximal region of each stented coronary vessel prior to sacrifice. The difference between these measurements is the neointimal area.

Histomorphometric analysis of stented arteries were used to determine the average cross sectional areas of the adventitial boundary layer (ABL), the external elastic lamina (EEL), the internal elastic lamina (IEL), the lumen (L), the adventitia (A), the media (M), the intima (I), the intima to media ratio (I/M) and an injury score. The injury score is the average of 36 determinations of injury consisting of twelve determinations in each of the proximal (a), middle (b) and distal (c) segments at the strut sites of the stented vessel. Injuries were scored either 0,1, 2 or 3 with 0 indicating an intact IEL (i.e. no injury) and 3 indicating a ruptured EEL with exposure to the adventitia (i.e. most severe injury).

Intramural, INFILTRATOR delivered ETC-216 treatment significantly reduced the intimal to media ratio in coronary vessels by 35 percent (LAD, LCX, and RCA combined). This effect was largely due to significant reductions of the I/M ratio of the RCA (-42%, p=0.002), LCX (-38%), and LAD (-29%). The histomorphometric data for all vessels (i.e. the RCA, LCX and LAD combined) and for vessel type (i.e. the RCA or the LAD) are shown graphically in Figures 5a and 5b, respectively.

We claim:

1. A method for treating or preventing before or during bypass surgery on diseased coronary, peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, or angioplasty, comprising administered locally at the site of injury to a vessel an effective amount of lipid modulating drug to prevent or reduce stenosis or restenosis or stabilize a plaque.

2. The method of claim 1 wherein the anti-proliferative drug is an alpha helical apolipoprotein or HDL associating protein.

3. The method of claim 2 wherein the apolipoprotein or HDL associated protein is selected from the group consisting of apolipoprotein A-I, apolipoprotein A-I Milano, apolipoprotein A-I Paris, apolipoprotein E, proapolipoprotein A-I, apolipoprotein A-II, proapolipoprotein A-II, apolipoprotein A-IV, apolipoproteins modified to include one or more sulfhydral groups, apolipoprotein C-I, apolipoprotein C-II, and apolipoprotein C-III, the alpha-helical sequences within these apolipoproteins, paraoxonase, cholesteryl ester transfer protein, LCAT and phospholipid transfer protein.

4. The method of claim 1 wherein apolipoprotein is apolipoprotein A-I Milano

5. The method of claim 2 comprising administering the apolipoprotein or HDL associated protein in combination with lipid.

6. The method of claim 5 wherein the apolipoprotein is administered in combination with lipid and HDL associated proteins.

7. The method of claim 5 wherein the ratio of

apolipoprotein to lipid by weight is between approximately 1:0.5 and 1:3.

8. The method of claim 1 wherein the lipid modulating agent is administered by an intramural infiltration device.

9. The method of claim 2 wherein the apolipoprotein or HDL associated protein is administered with a cathether.

10. The method of claim 1 wherein the apolipoprotein is administered in a gel.

11. The method of claim 4 wherein the apolipoprotein is apolipoprotein A-I Milano administered in combination with phospholipid in a ratio of between approximately 1:0.5 and 1:3, by weight in a dosage of between 0.05 and 0.3mg apolipoprotein A-I Milano/kg or between 4 and 6 mg apolipoprotein A-I Milano/vessel segment to be treated.

12. The method of claim 2 wherein the apolipoprotein or HDL associated protein is administered in a dose range between 0.01 mg apolipoprotein/kg and a dose limited by viscosity or device volume / coronary vessel segment.

13. The method of claim 1 wherein the apolipoprotein is administered in a dose range between 0.3 mg apolipoprotein/kg and a dose limited by viscosity or device volume / coronary vessel segment.

14. The method of claim 1 wherein nucleic acid molecules are delivered by an intramural infiltration.

15. The method of claim 14 wherein the nucleic acid molecules encode an alpha helical apolipoprotein or HDL associating protein.

16. The method of claim 14 wherein the nucleic acid molecules are oligonucleotides.

17. The method of claim 1 wherein the lipid modulating drug is administered in a single effective dosage.

18. The method of claim 1 wherein the lipid modulating drug is administered is multiple dosages.

19. The method of claim 1 further comprising systemically delivery a drug selected from the group consisting of anti-proliferative compounds, anti-inflammatory compounds, anti-hypertensive compounds, anti-coagulants, and lipid-regulating agents.

20. The method of claim 19 wherein the systemic therapy is begun before the local therapy.

21. The method of claim 19 wherein the lipid regulating agents are selected from the group consisting of niacin, statins, and fibrates.

22. The method of claim 19 wherein the anti-proliferative agents are selected from the group consisting of paclitaxel,rapamycin, AP-17 tirofiban, and abciximab.

23. The method of claim 19 wherein the agents that prevent or delay blood coagulation or platelet aggregation are selected from the group consisting of aspirin, IIb/IIIa inhibitors, clopidogrel, heparin and heparin fragments.

24. The method of claim 1 wherein the local delivery is through release from a catheter into the pericardial space.

25. The method of claim 2 wherein release is achieved through the use of coated stents.

26. The method of claim 1 to prevent or treat restenosis.

27. The method of claim 1 to stabilize plaque.

28. The method of claim 27 to reduce the consequence of plaque rupture including thrombus formation and ischemia.

29. A kit for treating or preventing before or during bypass surgery on diseased coronary, peripheral, and cerebral arteries, surgery to implant grafts or transplanted organs, angioplasty, or plaque stabilization comprising

means for prolonged local release an effective amount of lipid modulating drug to prevent or reduce stenosis or restenosis or stabilize a plaque.

30. The kit of claim 29 comprising an intramural infiltration device.

31. The kit of claim 29 comprising a catheter in combination with a reservoir for the apolipoprotein and/or lipid.

32. The kit of claim 29 comprising a means for administration of nucleic acid molecules.

33. The kit of claim 29 wherein the cholesterol lowering drug is selected from the group consisting of apolipoprotein A-I, apolipoprotein A-I Milano, apolipoprotein A-I Paris, apolipoprotein E, proapolipoprotein A-I, apolipoprotein A-II, proapolipoprotein A-II, apolipoprotein A-IV, apolipoproteins modified to include one or more sulfhydral groups, apolipoprotein C-I, apolipoprotein C-II, and apolipoprotein C-III, the alpha-helical sequences within these apolipoproteins, paraoxonase, cholesteryl ester transfer protein, LCAT and phospholipid transfer protein.

34. A stent coated with a material to be released at a site to be treated selected from the group consisting of alpha helical apolipoprotein or HDL associating protein alone or formulated with lipid, cells expressing the genes encoding alpha helical apolipoprotein or HDL associating protein, and naked DNA coding for alpha helical apolipoprotein or HDL associating protein for local delivery to an injury site.

Figure 1a Quantitative Coronary Angiography
LAD and RCA (combined)

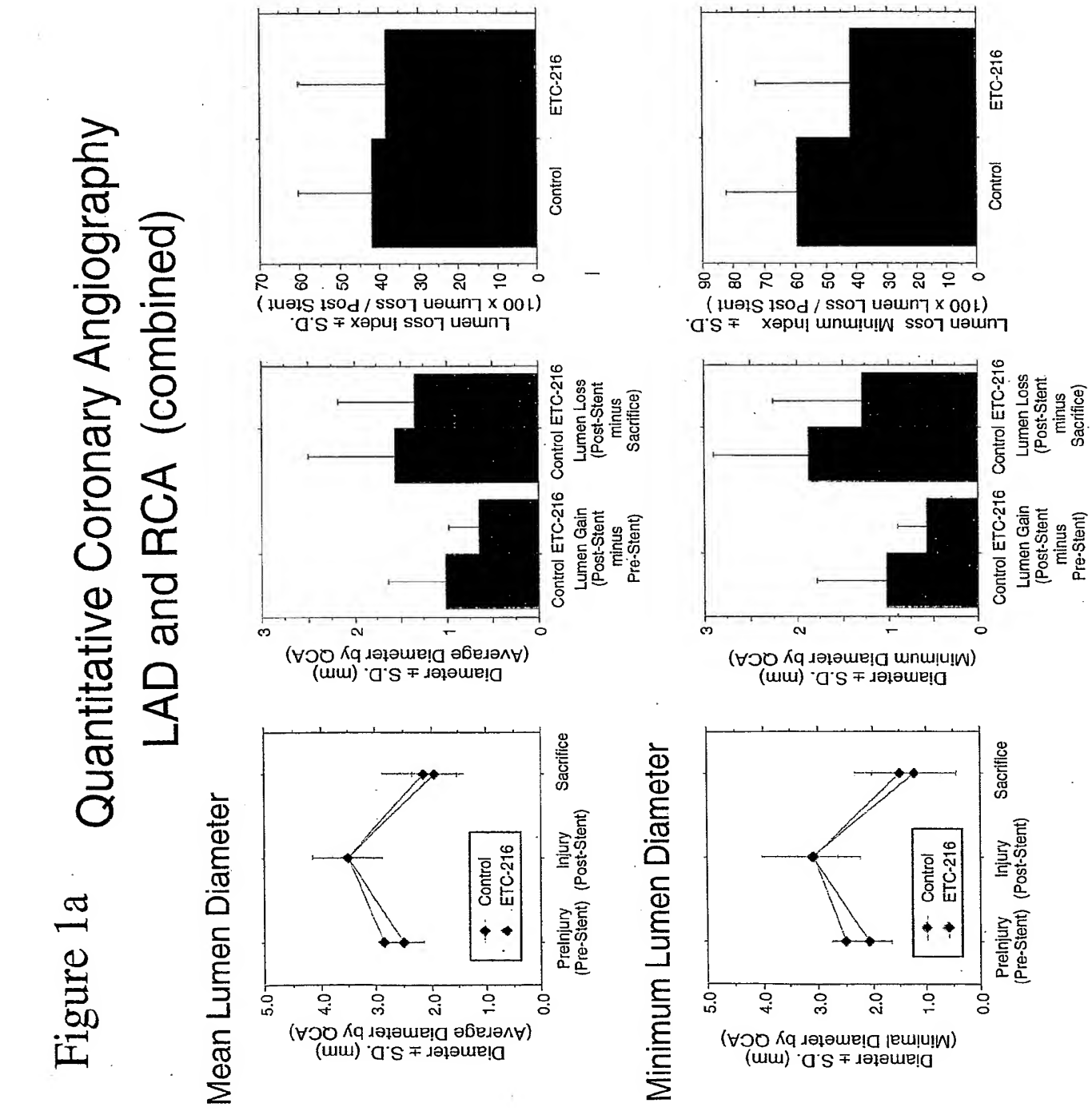

Figure 1b

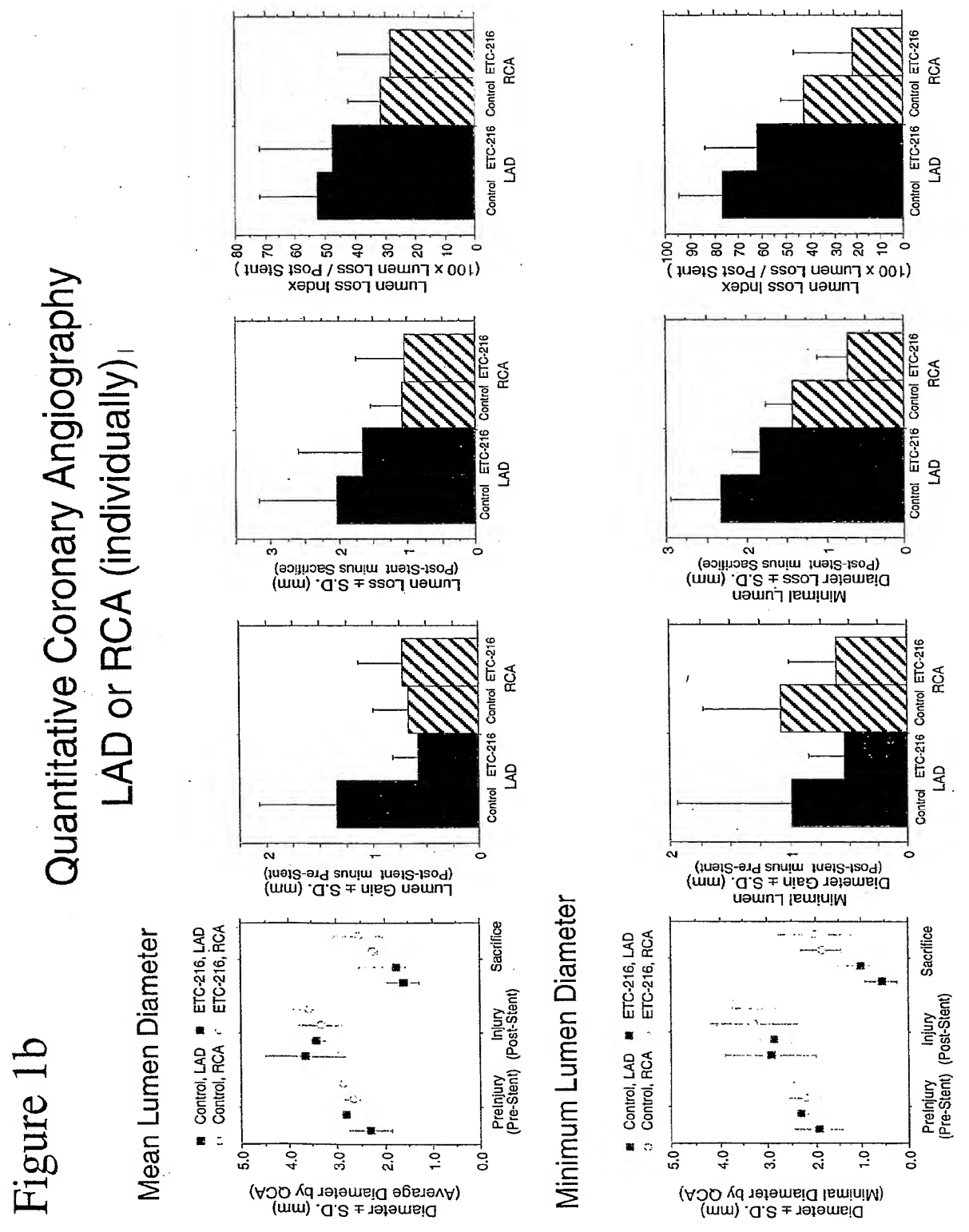
Quantitative Coronary Angiography
LAD or RCA (individually)
Mean Lumen Diameter
Minimum Lumen Diameter
Control, LAD
Control, RCA
ETC-216, LAD
ETC-216, RCA
Diameter ± S.D. (mm)
(Average Diameter by QCA)
(Minimal Diameter by QCA)
Preinjury (Pre-Stent)
Injury (Post-Stent)
Sacrifice
Lumen Gain ± S.D. (mm)
(Post-Stent minus Pre-Stent)
Minimal Lumen Diameter Gain ± S.D. (mm)
(Post-Stent minus Pre-Stent)
Lumen Loss ± S.D. (mm)
(Post-Stent minus Sacrifice)
Minimal Lumen Diameter Loss ± S.D. (mm)
(Post-Stent minus Sacrifice)
Lumen Loss Index
(100 x Lumen Loss / Post Stent)
Control ETC-216 Control ETC-216
LAD
RCA

Figure 2a

Effect of Single Dose ETC-216 Administration (100mg/kg) on IVUS Changes in Coronary Arteries in the Balloon Over-Inflated Stented Twenty-Eight Day Restenosis Pig Model

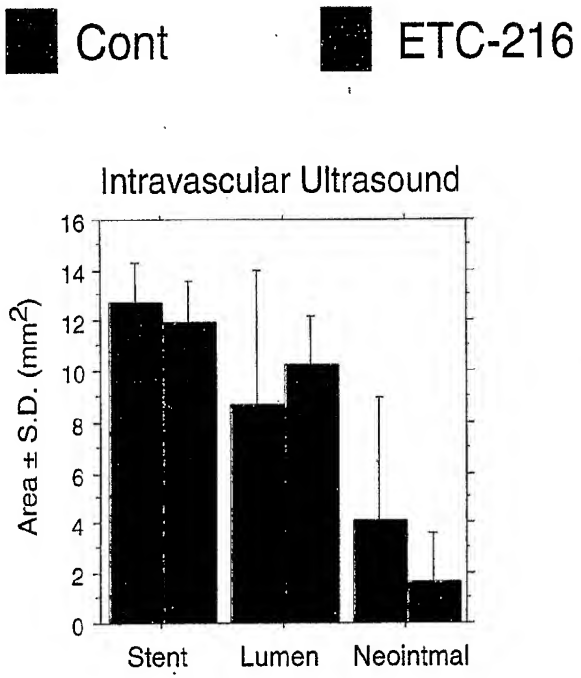

Figure 2b

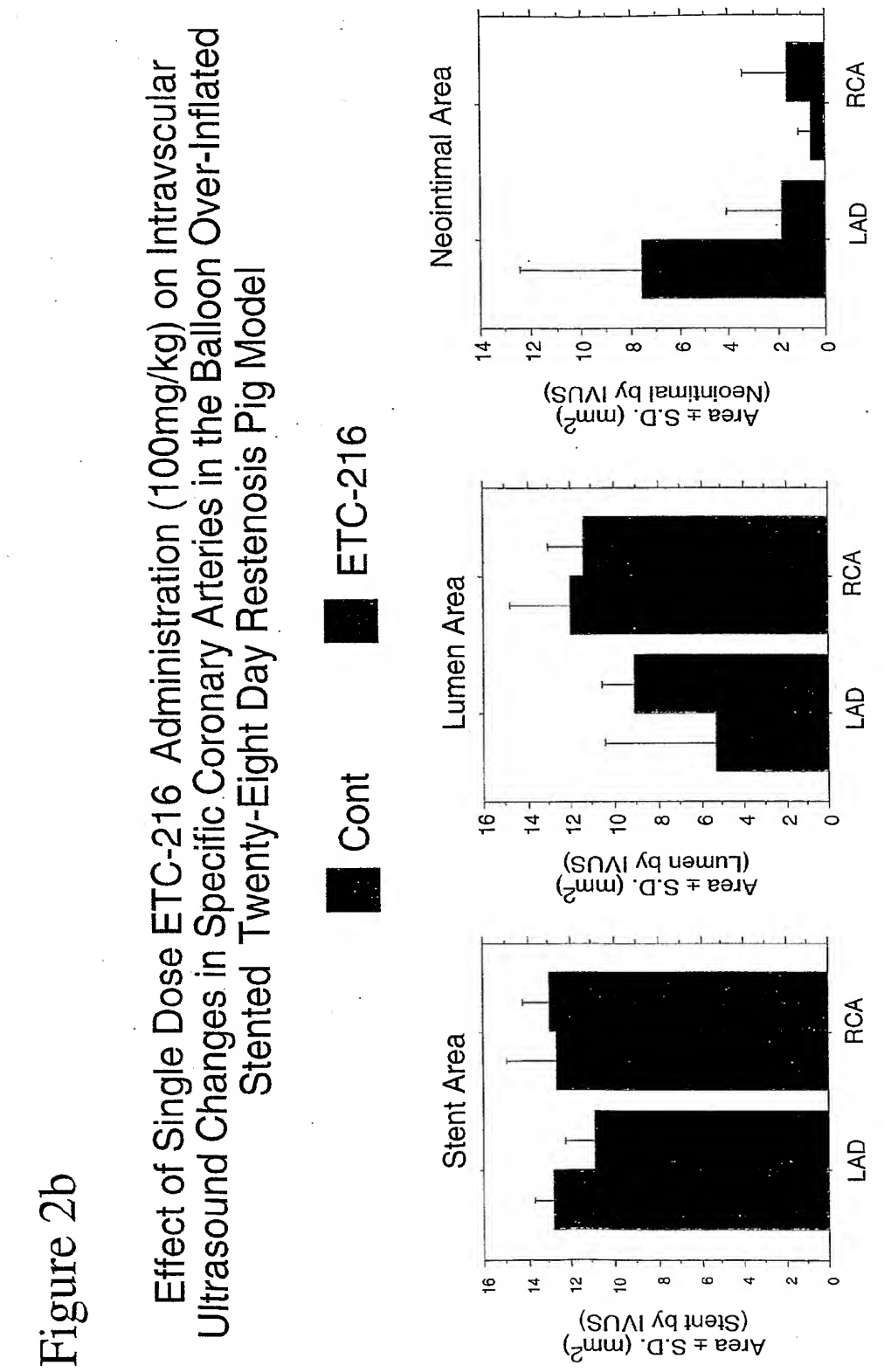
Effect of Single Dose ETC-216 Administration (100mg/kg) on Intravscular Ultrasound Changes in Specific Coronary Arteries in the Balloon Over-Inflated Stented Twenty-Eight Day Restenosis Pig Model
Cont
ETC-216
Stent Area
Area ± S.D. (mm²) (Stent by IVUS)
LAD
RCA
Lumen Area
Area ± S.D. (mm²) (Lumen by IVUS)
LAD
RCA
Neointimal Area
Area ± S.D. (mm²) (Neointimal by IVUS)
LAD
RCA

Figure 3a

Effect of Single Dose ETC-216 Administration (100mg/kg) on Histomorphometric Changes in Coronary Arteries in the Balloon Over-Inflated Stented Twenty-Eight Day Restenosis Pig Model

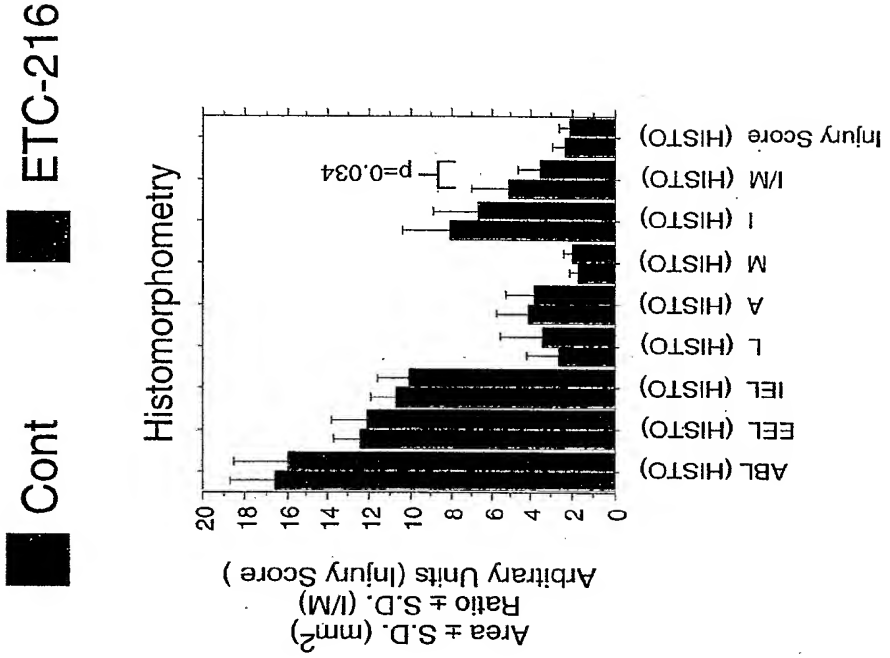

Figure 3b

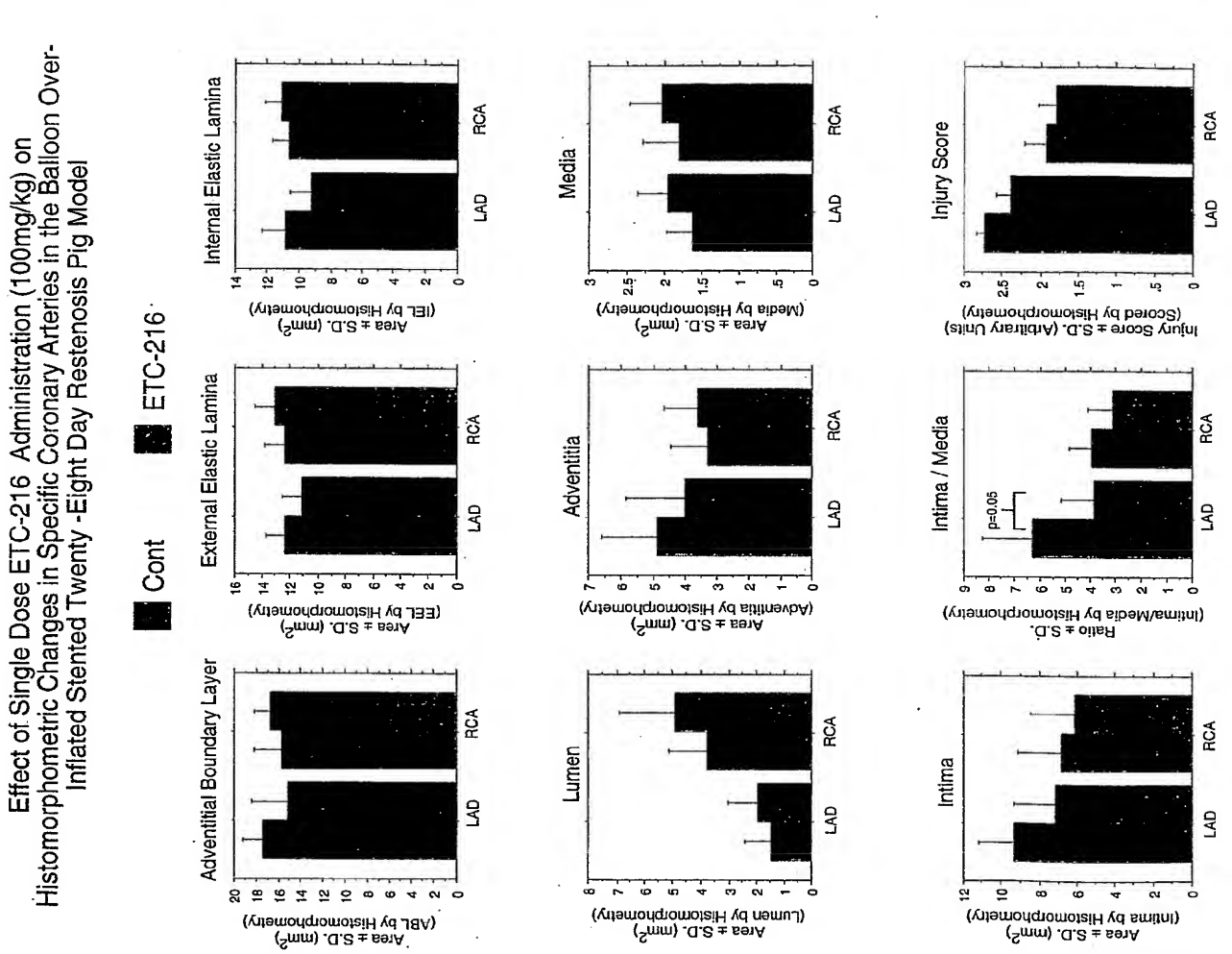
Effect of Single Dose ETC-216 Administration (100mg/kg) on Histomorphometric Changes in Specific Coronary Arteries in the Balloon Over-Inflated Stented Twenty -Eight Day Restenosis Pig Model
Cont
ETC-216
Adventitial Boundary Layer
Area ± S.D. (mm²) (ABL by Histomorphometry)
External Elastic Lamina
Area ± S.D. (mm²) (EEL by Histomorphometry)
Internal Elastic Lamina
Area ± S.D. (mm²) (IEL by Histomorphometry)
Lumen
Area ± S.D. (mm²) (Lumen by Histomorphometry)
Adventitia
Area ± S.D. (mm²) (Adventitia by Histomorphometry)
Media
Area ± S.D. (mm²) (Media by Histomorphometry)
Intima
Area ± S.D. (mm²) (Intima by Histomorphometry)
Intima / Media
Ratio ± S.D. (Intima/Media by Histomorphometry)
p=0.05
Injury Score
Injury Score ± S.D. (Arbitrary Units) (Scored by Histomorphometry)
LAD
RCA

Figure 4

Restenosis Correlation Variables of Coronary Arteries in Balloon Over-Inflated Stented Twenty-Eight Day Post-Injured Pigs Administered a Single Dose of ETC-216 (100mg/kg)

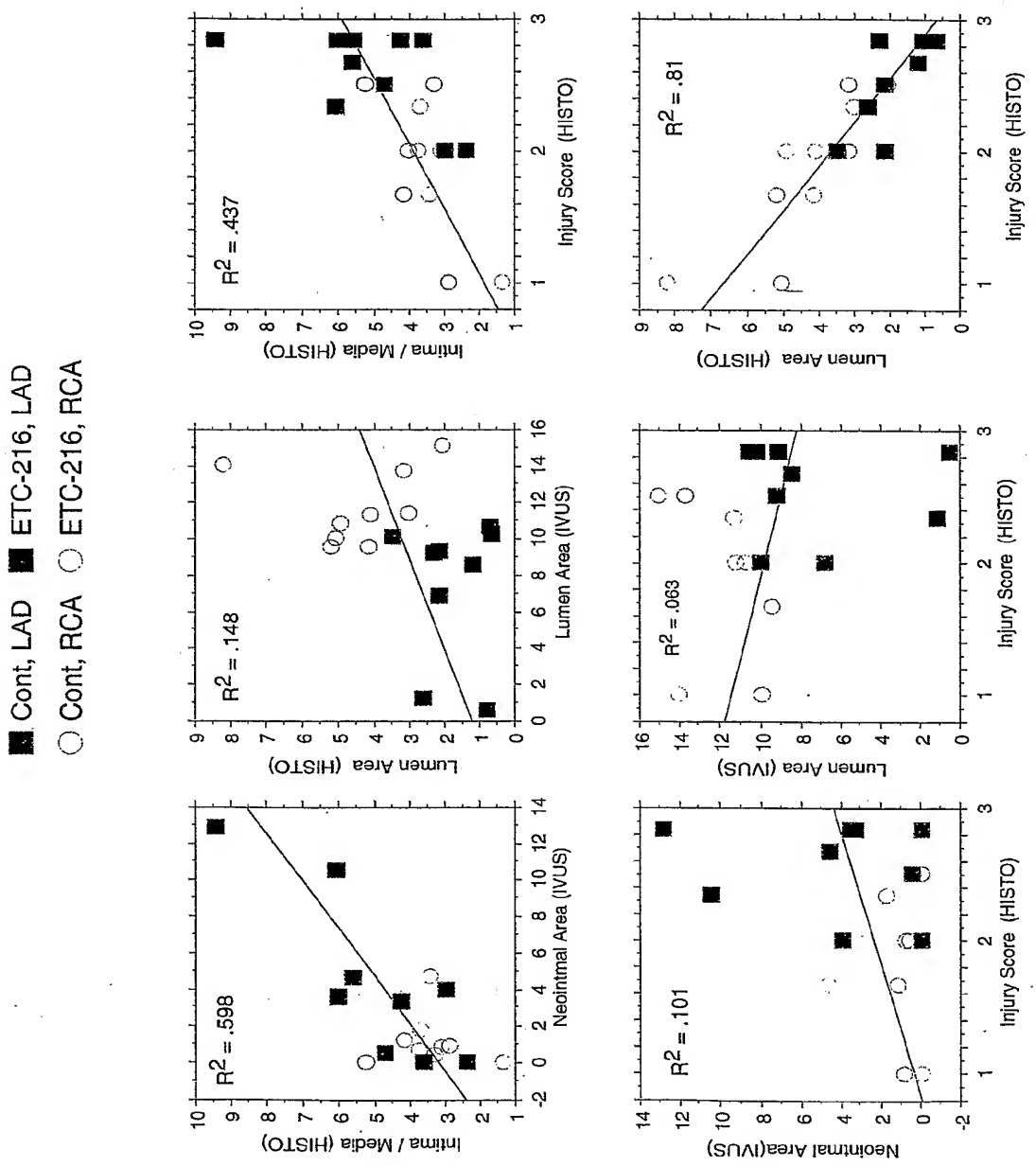

Figure 5a

Effect of Intramural Dose ETC-216 Administration on Histomorphometric Changes in Coronary Arteries in the Balloon Over-Inflated Stented Twenty-Eight Day Restenosis

Histomorphometry

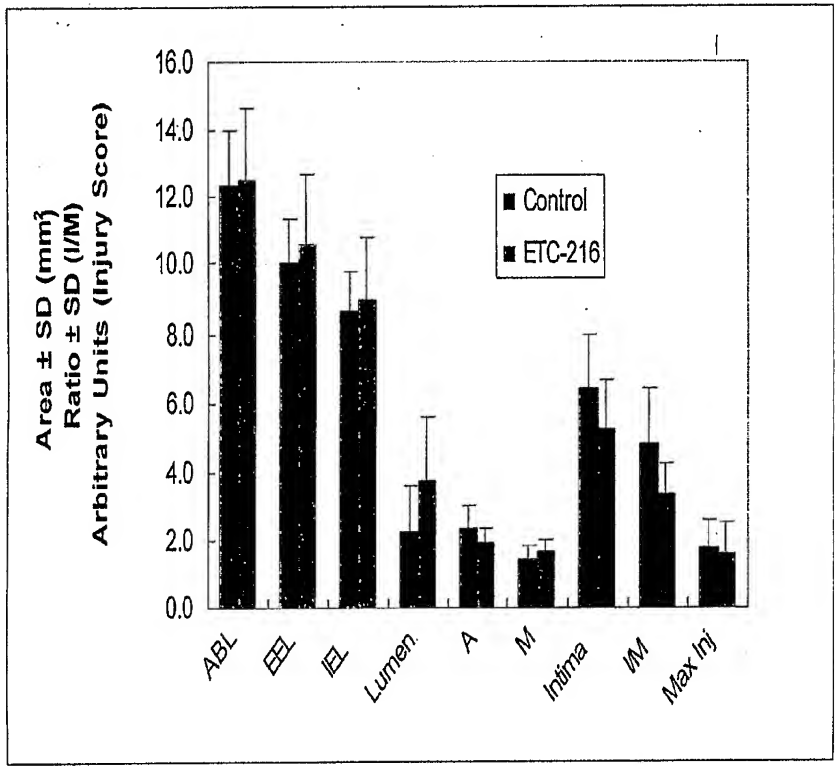

Figure 5b

Effect of Intramural Dose ETC-216 Administration on Histomorphometric Changes in Specific Coronary Arteries in the Balloon Over-Inflated Stented Twenty-Eight Day Restenosis Pig Model